AN INTERPRETATION OF SELF-STERILITY

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In certain hermaphroditic animals and plants, self-fertilization is often impossible. This gametic incompatibility has been called self-sterility. In the vegetable kingdom it is known to be comparatively widespread; in the animal kingdom, though it may be found later to be characteristic of many species, as yet only the Ascidian *Ciona intestinalis* has furnished material for study of the problem. (See Morgan,[1] Adkins, in Morgan,[2] and Fuchs.[3])

Ciona is not perfectly self-sterile. Individuals appear to vary in degree of self-sterility, though no case has yet been found where self-fertility is equal to cross-fertility. Morgan believes that there is a great difference in the compatibility of ova to sperm from other individuals, though Fuchs maintains that 100% of segmenting eggs can be obtained in every cross with normal ova if a sufficiently concentrated sperm suspension is used.

Fuchs has shown a chemical basis for the phenomenon by the difference in ease of cross-fertilization after contact of ova with sperm from the same animal and by the variation in ease of self-fertilization after certain artificial changes in the chemical equilibrium of the medium surrounding the ova, and by this work has brought the matter of self-sterility in Ciona in line with that in Angiosperms as worked out by Jost.[4]

Jost has shown that in the plants with which he worked only short tubes were formed after pollination with pollen from the same plant, though the necessary length of pollen-tube was easily developed after cross-fertilization. He saw as cause of these phenomena a chemotropism due to the presence of 'individueller Stoffe.' Pollen was indifferent to 'Individualstoff' from the same plant, but was stimulated by that from other plants.

To Correns[5] such an explanation of self-sterility seemed too general. He believed that a simple interpretation would account for the results he had obtained from *Cardamine pratensis*. Two plants B and G were crossed reciprocally and sixty of the offspring tested by pollinating from the parents, on the parents and from sisters. The back crosses of (B × G) or (G × B) with B and with G seemed to him to indicate four equal-sized classes with reference to gametic compatibility: (1) plants fertile

with both B and G; (2) plants fertile with B but not with G; (3) plants fertile with G but not with B; (4) plants fertile with neither B nor G.

These facts were interpreted by assuming the existence of two independently inherited factors that inhibit the growth of pollen-tubes. Representing these factors by the letters B and G, the original plants must be supposed to have had the formulae Bb and Gg respectively, since it is clear that type BB and GG could never be formed. When Bb is crossed with Gg the four types BG, Bg, bG and bg should result, of which the first three should be self-sterile. Plants BG should be fertile with plants bg, plants Bg should be fertile with bG and bg, plants bG should be fertile with Bg and bg, while plants bg should be self-fertile as well as cross-fertile with the other three classes. Attractive as this theory is, it is not clearly in accord with the facts. Plants of the type bg—inherently self-fertile—were not found, and the other classes showed many discrepancies.

Morgan[2] has offered another hypothesis that fits the data from both plants and animals. If I have not misunderstood the meaning of his rather general statement of the proposition, my own theory is only an extension of it, laid down perhaps a little more specifically. He says:

> The failure to self-fertilize, which is the main problem, would seem to be due to the similarity in the hereditary factors carried by the eggs and sperm; but in the sperm, at least, reduction division has taken place prior to fertilization, and therefore unless each animal was homozygous (which from the nature of the case cannot be assumed possible) the failure to fertilize cannot be due to homozygosity. But both sperm and eggs have developed under the influence of the total or duplex number of hereditary factors: hence they are alike; i.e., their protoplasmic substance has been under the same influence. In this sense, the case is like that of stock that has long been inbred, and has come to have nearly the same hereditary complex. If this similarity decreases the chances of combination between sperm and eggs we can interpret the results.

My own work has been done with the descendants of a cross between *Nicotiana forgetiana* (Hort) Sand., a small red-flowered species, and *Nicotiana alata* Lk. and Otto. var. *grandiflora* Comes, the large white-flowered sort commonly known as *Nicotiana affinis*. Both parents were undoubtedly self-sterile as over 500 plants of the F_1, F_2, F_3, and F_4 generations have been found to be self-sterile by careful tests.

Several experiments were made in which selfing, crossing *inter se*, and back crossing were done on a large scale, using plants of the F_2,F_3 and F_4 generations which had segregated markedly in size and were of

at least eight different shades of color. In the F_2 generation, twenty plants coming from two crosses between F_1 plants were selected for experiment. Each was selfed many times and in addition 131 inter-crosses were attempted, from four to twelve flowers being used in each trial. All attempts at selfing failed, while only two attempts at crossing were unsuccessful. Of the 129 successful inter-crosses, all but 4 produced full capsules, and it is probable that even this variability in ease of cross-fertilization was caused by attending conditions. One hundred and twenty other inter-crosses were made in the F_2 generation, with three failures.

In the F_3 generation, about one hundred inter-crosses were made between twelve plants which were the progeny of two sister F_2 plants. Six of these attempts failed.

In the F_4 generation, fifty-eight inter-crosses were made between ten plants that were the daughters of two F_3 plants. Fifty-three of these cross-fertilizations were successful.

Back crosses also were made in considerable numbers, though not to the extent one might desire. Plants A, B, C and D were combined in four different ways and among the plants resulting from these combinations eighty-five back crosses were attempted, of which five failed.

These facts will not fit any simple Mendelian formula similar to that proposed by Correns; furthermore, data from an experiment of a different kind appear to support Jost's idea of 'Individualstoffe' rather than Correns' idea of inhibitors. Pairs of plants were provided to furnish series of selfed and crossed flowers. The pistils of these flowers were fixed at regular periods after pollination, stained, sectioned and the pollen-tubes examined. Since the flowers on each plant had about the same length of pistils, curves of pollen-tube development for both crossing and selfing could be constructed. The pollen grains germinated perfectly on stigmas from the same plant, from 1200 to 2000 tubes having been counted in sections of single pistils. The difference between the development of the tubes in the selfed and the crossed styles was wholly one of rate of growth. The tubes in the selfed pistils developed steadily at a rate of about 3 mm. per twenty-four hours, with even a slight acceleration of this rate as the tubes progressed. If the flowers were sufficiently long-lived, one could hardly doubt but that the tubes would ultimately reach the ovules, though this would not necessarily mean that fertilization must occur. Since the maximum life of the flower is about 11 days, however, the tubes never traverse over one-half of the distance to the ovary. On the other hand, the tubes in the crossed pistils, though

starting to grow at the same rate as the others, pass down the style faster and faster, until they reach the ovary in four days or less.

From these facts it seems reasonable to conclude that the secretions in the style stimulate the pollen-tubes from other plants instead of inhibiting the tubes from the same plant.

The whole question, therefore, becomes a mathematical one, that of satisfying conditions whereby no stimulus is offered to pollen-tubes from the same plant, but a positive stimulus is offered to tubes from *nearly* every other plant.

The nearly constant rate of growth of pollen-tubes in the pistils of selfed flowers, compared with the regular acceleration of growth of the tubes from the pollen of other plants, undoubtedly shows the presence of stimulants of great specificity akin to the 'Individualstoffe' of Jost, though I believe their action to be indirect. Experiments by several botanists, which I have been able partially to corroborate, point to a single sugar, probably of the hexose group, as the direct stimulant. The specific 'Individualstoffe' I believe to reside in the pollen grains and to be in the nature of enzymes of slightly different character, all of which except the one produced by the plant itself for the use of its own pollen, or by another individual of the same genotype, can call forth secretion of the sugar that gives the direct stimulus. At least this idea links together logically the fact of the single direct stimulus and the need of 'Individualstoffe' to account for the results of the crossing and selfing experiments But whether or not this be the correct physiological inference, the crossing and selfing experiments call for an hypothesis that will account for no stimulation being offered the tubes from the plant's own pollen while at the same time great stimulation will be given the tubes from the pollen of *nearly* every other plant. This is a straight mathematical problem, and it is hardly necessary to say that it is insoluble by a strict Mendelian notation such as Correns sought to give. This is obvious to anyone familiar with the basic mathematics of Mendelism. On the other hand a near Mendelian interpretation satisfies every fact.

Let us assume that different hereditary complexes stimulate pollen-tube growth and in all likelihood promote fertilization, and that like hereditary complexes are without such effect. One may then imagine any degree of heterozygosis in a mother plant and therefore any degree of dissimilarity between the gametes it produces, without there being the possibility of a single gamete having anything in its constitution not possessed by the somatic tissues of the mother plant. From the chromo-

some standpoint of heredity the cells of the mother plant are duplex in their organization: they contain N pairs. The cells of the gametes contain N chromosomes, one coming from each pair of the mother cell; but they are all parts of the mother cell and contain nothing that that cell did not contain. These gametic cells cannot reach the ovaries of flowers on the same plant because they cannot provoke the secretion of the direct stimulant from the somatic cells of that plant.

All gametes having in their hereditary constitution something different from that of the cells of a mother plant, however, can provoke the proper secretion to stimulate pollen-tube growth, reach the ovary before the flower wilts, and produce seeds. Such differences would be very numerous in a self-sterile species where cross-fertilization must take place; nevertheless like hereditary complexes in different plants should be found, and this should account for the small percentage of cross-sterility actually obtained. It must be granted that this hypothesis satisfies the facts, but that is not all. It is admittedly a perfectly formal interpretation, but from a mathematical standpoint—granting the generality of Mendelian inheritance—it is the only hypothesis possible that can satisfy the facts.

In conclusion it should be mentioned that the cross-pollinated pistils show a considerable variation in the rate of growth of individual pollen-tubes, though our curves of growth have been made by taking the average rate of elongation. Is this variation a result of chance altogether or must one assume a differential rate of growth increasing directly with the constitutional differences existing between the somatic cells and the various gametes? If it is assumed that any constitutional difference between the two calls forth the secretion of the direct stimulus to growth, chance fertilization by gametes of every type different from that of the mother plant will ensue; if there is a differential rate, selective fertilization will occur. This question cannot be decided definitely at present, but two different lines of evidence point toward chance fertilization:

1. Flowers from a single plant pollinated by different males show no decided difference in rate of fertilization.

2. Color differences are transmitted to expected ratios.

Further, it will be recalled that beginning with the F_2 generation sister plants crossed together have given us our F_3 and F_4 populations, and that these F_3 and F_4 populations apparently have given a constantly increasing percentage of cross-sterility. This is what should be expected under the theory that a small difference in germ plasm constitution is as active as a great difference in causing the active stimulation to pollen tube

growth. Breeding sister plants together in succeeding generations causes an automatic increase of homozygosity as is well known. This being a fact, cross-sterility should increase. Such an increase in cross-sterility has been observed in the F_3 and the F_4 generations, but it would not be wise to maintain dogmatically that it is significant.

[1] Morgan, T. H., Some further experiments on self-fertilization in Ciona. *Biol. Bull.*, 8, 313–330 (1905).

[2] Morgan, T. H., *Heredity and Sex.* New York. Columbia University Press, ix + 1–282 (1913). (page cited 217).

[3] Fuchs, H. M., On the conditions of self-fertilization in Ciona. *Archiv. f. Entwickl. d. Org.*, 40, 157–204 (1914); The action of egg-secretions on the fertilizing power of sperm. *Archiv. f. Entwickl. d. Org.*, 40, 205–252 (1914).

[4] Jost, L., Zur Physiologie des Pollens. *Ber. d. deut. Bot. Ztg., Heft V and VI* (1907).

[5] Correns, C., Selbststerilität und Individualstoffe. *Festschr. d. med. nat. Gesell. zur* 84. *Versamml. deutsch. Naturforscher u. Arzte. Münster i. W.*, pp. 1–32 (1912).

THE BASAL CALORIC OUTPUT OF VEGETARIANS AS COMPARED WITH THAT OF NON-VEGETARIANS OF LIKE WEIGHT AND HEIGHT

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Among the numerous, more or less scientifically attested, facts concerning the influence of a vegetarian diet are statements relative to an observed increase in endurance and the belief that vegetarians live upon a somewhat lower metabolic plane than do flesh eaters, who, it is asserted, are unduly stimulated by the protein in their food. The measure of the basal gaseous metabolism, which may be considered as the carbon-dioxide production and oxygen consumption during complete muscular repose and in the post absorptive condition, that is, at least 12 hours after the last meal, gives an admirable index of the metabolic activity.

We have been able to make observations on the basal gaseous metabolism of 11 men and 11 women, who had subsisted upon a vegetarian diet for a considerable period of time, extending, on the average, over several years. With the subjects in the post absorptive condition and lying quietly upon a comfortable couch, the total carbon-dioxide production and oxygen consumption of each of these individuals were measured on several days by means of the universal respiration apparatus. As a rule the values obtained agree well with each other and the averages